PROCEDURES MANUAL,
AQUATIC BIOASSAY SERVICE PROJECTS

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AQUATIC BIOLOGY GROUP

ANIMAL SCIENCES WING

ALBERTA ENVIRONMENTAL CENTRE

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SUMMARY

The purpose of this report is to serve as a reference and training guide for staff involved in aquatic bioassays at the Alberta Environmental Centre. The manual describes the water treatment system, procedures for acquisition and maintenance of fish, and bioassay methods.

I. INTRODUCTION

The Aquatic Biology Group engages in environmental research which requires the use of rainbow trout and other indigenous aquatic species as test organisms. In addition, there is a service committment to Alberta Environment Water Quality Control Branch, which is to conduct 96 h acute lethality rainbow trout bioassays on industrial effluents. Data from these tests are used by regulatory and licensing agencies of Alberta Environment to issue operating licenses which regulate the concentrations of chemicals discharged in wastewater.

Because of the magnitude of research and service work performed, it is necessary to maintain large stocks of fish at the Centre. The purpose of this manual is to explain the procedures for acquisition and maintenance of fish. In addition, procedures for conducting 96 h acute lethality determination rainbow trout bioassays, and a description of our water treatment system are included.

II. WATER QUALITY

A constant supply of treated water, meeting the criteria outlined in Table 1, is needed to carry out most of the aquatic research and service programs. The projected water demand is approximately 60 IGPM (Imperial Gallons per Minute) or 272 L min^{-1}, of which 50 IGPM or 227 L min^{-1} is required by Aquatic Biology. Due to a limited water supply in the Town of Vegreville it was necessary to design a 90% water recycle system for the Centre. The elevated levels of iron, zinc and copper in the town water make it unsuitable for direct use for holding aquatic organisms (Table 2) prior to treatment.

By 1983 City of Edmonton water will be piped to Vegreville. The system design described here will accomodate the change-over with minimum modifications.

Table 1. Recommended Water Quality for Rearing Aquatic Organisms.

pH	7.0 - 8.5
Specific conductance	100 - 200 μ siemens cm^{-1}
Chemical oxygen demand	<10 mgL^{-1}
Boron	<100 μgL^{-1}
Flouride	<100 μgL^{-1}
Un-ionized ammonia	<20 μgL^{-1}
Total Dissolved:	
Aluminum	<1 μgL^{-1}
Arsenic	<1 μgL^{-1}
Chromium	<1 μgL^{-1}
Cobalt	<1 μgL^{-1}
Copper	<1 μgL^{-1}
Iron	<30 μgL^{-1}
Lead	<1 μgL^{-1}
Nickel	<1 μgL^{-1}
Zinc	<1 μgL^{-1}
Residual Chlorine	<0.5 μgL^{-1}
Cadmium	<0.1 μgL^{-1}
Mercury	<100 ngL^{-1}
Silver	<100 ngL^{-1}
Total organophosphorous pesticides	<50 ngL^{-1}
Total organochlorine pesticides plus polychlorinated biphenyls	<50 ngL^{-1}
Suspended solids	<20 mgL^{-1}
Total organic carbon	<10 mgL^{-1}

Water quality is considered constant if the hardness, alkalinity, and specific conductance vary by less than 10% in their respective averages and the monthly range is less than 0.4 units.

Source: American Public Health Assoc. (1975).

Table 2. Physical and Chemical Parameters of the Domestic Water Supply, Vegreville. Feb/77 - March/81

Chemical Parameter	Mean	Range	Concentrations expressed as MgL^{-1} No. of Samples Analyzed
pH	7.9	7.4 - 8.5	6
Iron	0.72	0.05 - 5.83	6
Calcium	56.0	35.0 - 156.0	6
Hardness Total	224.0	127.0 - 562.0	6
Potassium	9.9	10.0 - 22.0	6
Fluoride	0.86	0.12 - 1.09	6
Sulfate	289.0	165.0 - 573.0	6
Alkalinity Total	204.0	106.0 - 545.0	6
Biological Oxygen Demand	--	<1.0 - 9.0	6
Chemical Oxygen Demand	46.5	15.0 - 88.0	6
Carbon Total	58.0	40.0 - 67.0	6
Total Residue (calculated)	741.0	520.0 - 834.0	6
Non-filterable Residue	8.0	4.0 - 15.0	6
Ammonia	--	<0.02 - 0.21	6
Nitrogen Total Kjeldahl	1.18	0.77 - 1.39	6
$NO_2 + NO_3$	--	<0.050 - 0.303	6
Phenols	0.006	0.003 - 0.009	6
Odor	1	-----	6
$Chromium^{+6}$	--	<0.001 - <0.002	5
Color T_2	96	95 - 96	2
Cyanide	--	<0.002 - 0.004	6
Arsenic	--	<0.0002- 0.0010	6
Antimony	--	<0.0005- 0.0014	6
Copper	0.207	0.143 - 0.270	6
Cadmium	<0.001	-----	6
Zinc	0.257	0.001 - 1.780	6
Chromium	--	<0.001 - 0.008	5
Vanadium	<0.002	-----	6

Table 2. continued. Concentrations expressed as MgL^{-1}

Chemical Parameter	Mean	Range	No. of Samples Analyzed
Silver	<0.001	-----	4
Conductivity	990.0	657.0 -1820.0	6
Total Dissolved Solids	639.0	399.0 -1360.0	6
Magnesium	21.0	9.0 - 42.0	6
Sodium	135.0	85.0 - 250.0	6
Silica	5.8	1.0 - 18.8	6
Chloride	11.0	6.0 - 17.0	6
Bicarbonate	253.0	168.0 - 664.0	6
Oil & Grease	--	<0.2 - 0.4	5
Total Organic Carbon	8.0	2.0 - 13.0	6
Total Inorganic Carbon	50.0	34.0 - 57.0	6
Turbidity	4.3	1.0 - 15.0	5
Phosphorous Total	0.144	<0.02 - 0.255	6
Phosphate Dissolved Ortho	0.018	0.004 - 0.032	6
Nitrite	--	<0.001 - <0.1	6
Surfactants	0.20	0.16 - 0.27	6
Tannins & Lignins	--	<0.1 - 0.21	2
Color	95.0	91.0 - 97.0	2
Mercury	<0.0001	-----	6
Selenium	<0.0002	-----	6
Cobalt	--	<0.001 - 0.003	6
Nickel	0.011	0.003 - 0.055	6
Lead	--	<0.003 - 0.013	6
Manganese	0.224	0.075 - 0.345	6
Beryllium	<0.001	-----	4
Molybdenum	--	<0.001 - 0.009	6
Aluminum	0.530	0.106 - 1.800	6

A. WATER TREATMENT SYSTEM

The water treatment system, located in the General Services Building, consists of a primary treatment system to improve the quality of incoming water and a secondary treatment system to upgrade the recycled water.

1. Primary Treatment System

The primary treatment system, capable of treating 30 IGPM or 136.5 L min^{-1}, consists of three identical modules, each capable of treating 10 IGPM or 45.5 L min^{-1}. Twenty IGPM or 91.0 L min^{-1} is used to provide water for the recycle system and 10 IGPM or 45.5 L min^{-1} is used to meet other Centre needs.

The system is designed in modular form to insure that a shut-down of the entire system will not occur during equipment failure or service. One primary treatment module (Figure 1) consists of the following equipment:

a) 30 IGPM (136.5 L min^{-1}) ultra-violet dechlorinator sterilizer: completes sterilization and reduces residual chlorine.

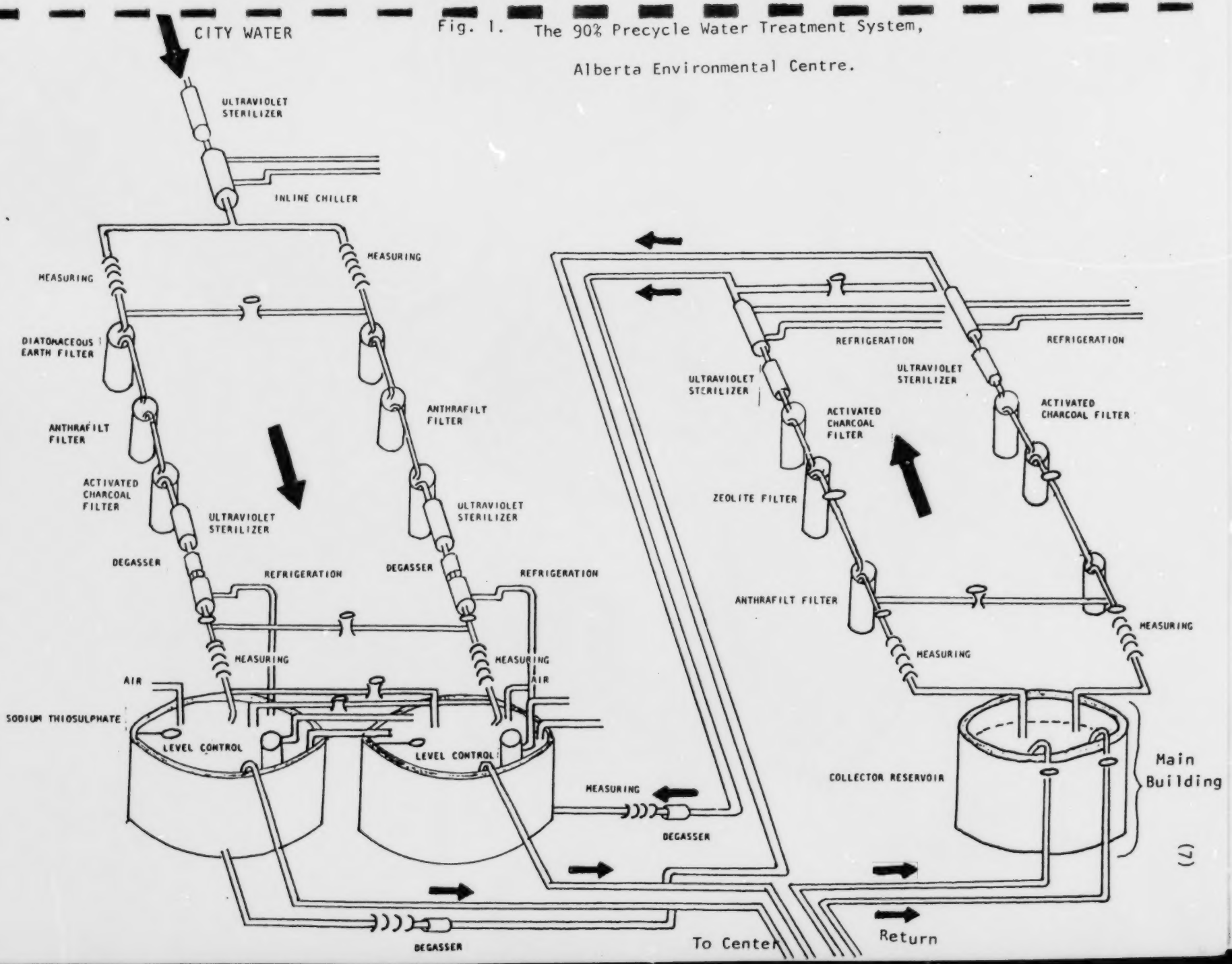
b) Inline chiller: maintains the water temperature at 10°C. This ensures a constant temperature in the primary treatment system, thus keeping the efficiency of the system constant.

c) 20 IGPM (91.0 L min^{-1}) diatomaceous earth filter: reduces suspended solids, and to some degree heavy metals.

d) 10 IGPM (45.5 L min^{-1}) anthrafilt filter: removes suspended solids down to 10 µm from the water.

Fig. 1. The 90% Precycle Water Treatment System,

Alberta Environmental Centre.



(7)

e) 10 IGPM (45.5 L min^{-1}) activated charcoal filter: removes chlorine down to 0.01 ppm, chloroamines, phenolics and to some extent copper.
f) 10 IGPM (45.5 L min^{-1}) ultra-violet dechlorinator sterilizer: further reduces remaining residual chlorine levels, by 50% to 0.005 ppm, and sterilizes the water, eliminating fish pathogens before the water is stored or diverted to other Centre requirements.
g) Degasser: reduces dissolved oxygen and nitrogen levels to saturation or slightly above saturation.

All the filters are equipped with automatic backwash systems controlled by a monitoring system.

Treated water intended for aquatic facilities goes into one of three 3125 IG (14,218.75L) insulated fiberglass reservoirs. The reservoirs are equipped with 3 HP refrigeration units and stainless steel coils which maintain the temperature at 10°C. The water is then pumped to the aquatic holding facilities. Some of the water is directed back to the reservoirs to mix the water in the tanks.

Treated water is available to individual laboratories in the Aquatic Biology area. Depending upon needs, stills will be required to treat water, in addition deionizing columns will be used to supply a higher quality water to laboratories. This water is supplied by the third primary treatment module.

2. Secondary Treatment System (Recycle)

Water from the aquatic holding facility passes through a screen or separator to remove fecal matter and food particles and is collected in a common reservoir. Water is then pumped back to the treatment area, with 10% of the water going to waste.

The secondary treatment system consists of two identical modules, with the capability of a third being added. It consists of the following equipment (Figure 1):

a) 30 IGPM (136.5 L min^{-1}) anthrafilt filter: reduces suspended solids down to 10 μm.

b) 50 IGPM (227.5 L min^{-1}) zeolite filter: removes ammonia from the water.

c) 30 IGPM (136.5 L min^{-1}) activated charcoal filter: further reduces suspended solids and removes noxious gases and nitrogen compounds.

d) 30 IGPM (136.5 L min^{-1}) ultra-violet dechlorinator sterilizer: kills bacteria and prevents possible fish pathogens from contaminating the water reservoirs.

e) Degasser: reduces dissolved oxygen and nitrogen to near saturation levels. The upgraded water, approximately 50 IGPM (227.5 L min^{-1}) is returned to one of the three reservoirs.

All of the filters are equipped with automatic backwashers controlled by the monitoring system.

3. Monitoring System

An automatic sequential backwashing system was designed to monitor the primary and secondary water treatment system, thus insuring maximum efficiency. Backwashes occur in sequence to minimize water used and to insure continuous operation of the systems. The following parameters are measured at the beginning and end of each system: dissolved oxygen, conductivity, pH, temperature and line pressure. Maximum and minimum acceptable limits are programmed into each unit, and an alarm notifies personnel if any of these limits are exceeded. In the future, the

system will be monitored by computer and analytical data on the parameters will be stored for data evaluation.

III. ACQUISITION

The protocol for acquisition of fish for research purposes encompasses a broad range of categories which are essential to provide high quality experimental results.

A. SPECIES SELECTION

Hatchery reared fish have been chosen over wild fish for toxicological research because they have a known history. In addition, disease, diet, spawning times and rearing water composition are also known (Brauhn and Schoettger, 1975).

The salmonids are among the most sensitive species to many toxicants (Stephan, 1975). Rainbow trout (Salmo gairdneri Richardson) are the standard test species used by the Aquatic Biology Group. The species has attained world-wide use to represent the cool-water salmonid family (Sprague, 1973) and is the most widely cultured salmonid for sport fishing (Bardach, Ryther & McLarney, 1972). In addition rainbow trout are easy to raise in captivity and are relatively tolerant of different temperatures, salinities, and population densities (Bardach, Ryther & McLarney, 1972).

1. Indigenous Species

In the future, indigenous species may be cultured at the Alberta Environmental Centre. Many of the freshwater fish common to the lakes and streams of the Americas may be induced to spawn in the laboratory,or collected when in a gravid state (National Academy of Sciences, 1974).

Standard culture techniques are available for most species, while some may require methods development.

B. SOURCE OF FISH

Although eggs are relatively easier to ship and treat with chemicals, fingerlings will also be purchased depending upon the needs of the group.

All movements of fish into Canada or between provinces require a permit. This permit is issued only to producers whose facilities have been inspected and certified. The "Regulations are designed to prevent the spread of infectious diseases through inspection of production sources of fish stocks, and to control the movement of infected fish stocks. They apply to live cultured fish, eggs of fish, and dead products of cultured fish destined to move into Canada or across provincial boundaries within Canada. In the event of violation of these Regulations, seizure and other powers of the Fisheries Act apply." (Fish. Environ. Canada, 1976) (Appendix A).

Fish should be available throughout the year in a range of small uniform sizes. This requires communication among the researcher, culturist and the supplying hatchery. At present fish are purchased from hatcheries in Ontario or the United States (Table 3). The Fish and Wildlife Division of Alberta Energy and Natural Resources is expanding its hatchery operations and should be able to supply the Aquatic Biology Group with rainbow trout when facilities at the Alberta Environmental Centre are fully operational.

Table 3. Sources of Rainbow Trout Fry and Eggs

Hatchery	Address	Phone#	Contact Person
Goosen's	RR#2, Otterville, Ontario	(519)879-6854	Mr. Rene Goosen
Spring Creek Hatchery	Upper Spring Creek Lewistown, Montana	(406)538-3538	Mr. Bob Von Lindern
Mount Lassen Trout Farms	Route 5, Box 36 Red Bluff, California	(916)597-2222	Mr. Phil Mackey
Fish and Wildlife (potential)	Raven Hatchery Caroline, Alberta	(403)427-6730	Mr. Terry McFadden

C. FISH ACQUISITION

The purchase of fish requires that an importation permit be obtained from Fish and Wildlife and copies be sent to the supplier. The permit is valid for a specified period of time, usually one week, during which the fish must arrive.

D. TRANSPORT

It may be necessary to transport live fish, either from the airport or as a result of field collection. Truck transport is a reliable and an economic method of moving fish for short distances (Brauhn and Schoettger, 1975).

The shipping container is generally a thick walled styrofoam cooler with a close fitting lid, lined with a polyethylene bag. A styrofoam cooler is chosen because it is lightweight with good insulating properties, and small enough to be handled by one person (National Academy of Sciences, 1974).

Primary hazards involved in transport are: oxygen deficiency, excess carbon dioxide and ammonia, and adverse temperatures. Mechanical stress due to overcrowding and rough handling may also present a potential problem. If possible, the fish are not fed for 48 hours prior to shipping, and the water temperature is gradually lowered to 3°C 24 hours before shipping (Brauhn and Schoettger, 1975).

A new clear polyethylene bag is rinsed twice with the water that the fish will be shipped in to remove toxic residues. If the fish are shipped from a laboratory, then dilution water is used; if they are collected in the field, ambient water is used. The bag is filled with 4- 5 L of water (National Academy of Sciences, 1974). Loading should

not exceed 454 g (1 lb) per 3.8 L (1 gal.) (Brauhn and Schoettger, 1975). The bag is then collapsed to remove excess air, partially inflated with pure oxygen, and sealed. The bag is placed in a cooler with ice, and the top is securely fastened. The cooler should be placed in a sturdy corrugated cardboard box.

E. RECEIPT OF FISH AND ISOLATION

All incoming fish must be maintained in the isolation laboratory for two weeks before being introduced into the holding laboratory. Each isolation tank will be provided with separate color-coded dip nets, feeding and cleaning supplies, and any other equipment needed during the holding period. To minimize the possibility of disease transmission, there will be no transfer of fish or water among tanks. Instruments will not be moved from the isolation laboratory. Equipment must be disinfected between tanks (See Section IV Maintenance).

Procedures to follow upon receipt of fish will depend upon the mode of transportation used in shipping.

1. Air-shipped Fish

Because fish are presently shipped by air they will be subject to some stress. Dissolved carbon dioxide (carbonic acid), and ammonia will accumulate, which may affect pH and inhibit gas exchange during respiration. In addition, oxygen concentration in water decreases as shipping time increases. Ammonia is the main nitrogenous end product of fish and is excreted by the kidneys and gills. It is highly soluble in water and exists as NH_4^+, NH_3 or NH_4OH. At pH <7, most of the ammonia is present in the ionized form (NH_4^+), while at pH 8 or greater the

level of the unionized form (NH_3) becomes more significant. Greater than 0.2 mgL^{-1} NH_3 is undesirable for rainbow trout (National Academy of Sciences, 1974).

Fish which are air-shipped will arrive at a low temperature, around 3°C (Brauhn and Schoettger, 1975); hence temperature acclimation should be a prime consideration. In the laboratory, the bags of fish should be gently removed from the boxes and floated on the surface of the receiving water until the temperatures are equalizd at 10°C (the temperature of the isolation tanks). At this time dilution water may be added to the bags at a rate of about 50 ml min^{-1} (Brauhn and Schoettger, 1975). When the water in the bags has been diluted by approximately 50% the fish may be gradually eased into the receiving water.

2. Truck-transported Fish

Temperature acclimation upon receipt of fish should follow procedures described above. Accompanying truck-transported fish should be a chemical analysis of the hatchery water pH, alkalinity, hardness and temperature. If there is an extreme difference between the hatchery water and the receiving water, dilution water should be added to the bags at a rate of about 20 ml min^{-1}. If no stress is observed when the water in the bags has been diluted by approximately 50%, the fish may be gradually eased into receiving water. If the fish are stressed it will be necessary to acclimate them at a slower rate. Normally, temperature change should not exceed 1° day^{-1} (Brauhn and Schoettger, 1975).

3. Isolation

A fish inspection program will begin when the fish first arrive at the Centre, and will continue until they leave. The intent of this program is to provide healthy fish stocks for experimental work. Data will be used to determine the suitability of the fish stocks. Fish will be held in the isolation room for two weeks. During this time they will be observed for stress, disease and parasites. Immediately upon receipt, 5 - 10 slides will be prepared from skin, gills and gastrointestinal tracts of a sample of fish, both moribund and normal. The slides will be examined under the light microscope for ecto and endo - parasites by Pathology and Aquatic Biology staff. Deformed fish and fish with lesions will be killed and examined histopathologically and bacteriologically. All fish will be treated with oxytetracycline to remove existing flora and fauna, and allow new flora and fauna to develop which will be adapted to the laboratory dilution water. Microscopic examination of skin, gills and gastrointestinal tract of a sample of fish should be repeated on the tenth day of isolation (Brauhn and Schoettger, 1975). This is to ensure that the fish will be ready to move into the fish holding laboratory. In order to maintain a performance record of mature fish, nose tags or freeze-branding will be used to mark the fish. This method may also be used for special projects where small populations of fish will be used. Water used in the isolation room will not be recycled.

F. ACCLIMATION

At the end of the holding period, fish will be introduced into the fish holding area, where they will be held in fiberglass insulated

tanks. Fish will be observed for disease and stress, (Section IV Fish Maintenance), and distributed to experimental laboratories as required. Fish will be acclimated to 15°C in acclimation tanks at a rate of 1°C day $^{-1}$ (Brauhn and Schoettger, 1975).

G. RECEIPT OF EGGS AND INCUBATION

Generally "eyed" eggs will be purchased by the Aquatic Biology Group. Eggs are "eyed" as soon as the eye of the embryo is clearly visible, approximately two to three weeks after fertilization. Eyed eggs are chosen because they are relatively tolerant of handling. Newly eyed eggs are preferable as they are less likely to hatch prematurely due to high temperatures enroute (Bardach, Ryther and McLarney, 1972). Eggs will be directed to the incubation laboratory when they are received.

Prior to shipping, egg temperature is gradually reduced to 1°C. The eggs are packed in trays similar to incubation trays, and wrapped in cheesecloth. Eggs must be kept cool and moist, but are not covered in water which would require aeration to prevent asphyxiation (Bardach, Ryther, and McLarney, 1972). The eggs are then placed in a well insulated box with an ample supply of ice.

Upon receipt, the eggs are removed from the shipping container and sorted. They are left in the shipping trays, and treated water at 12°C is added at a rate to limit the egg temperature increase to 2°C h^{-1} (Brauhn and Schoettger, 1975). The egg tray is floated in 12°C water to prevent rapid warming from the air. When incubation temperature is reached, surface disinfection of the eggs for bacteria and fungus will take place using a 100 ppm Bridine solution. The eggs are exposed for

10 minutes and rinsed (Papst, 1981). The water used is not recycled. The eggs are then placed in an incubation tray.

Eggs are incubated in a vertical-flow eight tray incubation cabinet constructed of non-toxic fiberglass. The incubator contains a series of fiberglass trays stacked so that any individual tray may be totally or partially removed. Water enters at the top of the incubator and is piped from tray to tray so that it enters each tray at the bottom. This provides aeration through the upwelling of the water.

Optimal hatching occurs in subdued lighting in gently flowing water with a temperature of 8-13°C, and a dissolved oxygen concentration of at least 7 mg L^{-1}. The rate of hatching is determined by temperature (Table 4) (Bardach, Ryther and McLarney, 1972). Disease free stock will be kept in the incubation laboratory in 8' (2.44 m) rearing troughs until the fish are large enough to be moved to the holding area.

Table 4. Hatching Rate of Eggs of Rainbow Trout at Different Temperatures

Temperature (°C)	Days to Hatching
4.5	80
7.3	48
10.0	31
12.0	24
15.7	19

Source: Adapted from Bardach, Ryther and McLarney, 1972.

IV. FISH MAINTENANCE

There are many factors which must be considered to maintain fish in good condition for an extended period of time in the laboratory. These include loading, nutrition and feeding, lighting, routine maintenance and behavior.

A. LOADING.

The loading capacity or number and weight of fish that can be maintained in good condition in tanks (kg.fish.m^{-3} water), depends upon metabolic requirements, i.e. the size of fish, dissolved oxygen, feeding rate, temperature, and rate of water exchange.

There are at least seven methods to determine loading capacity (Phillips, 1970 and Klontz, Brock and McNair, 1978). We have selected a method presented by Speece, (1973). Speece constructed a nomograph (based on production requirements) to predict water flow requirements per kilogram of fish to limit ammonia to 0.5 mg L^{-1}, as a function of length for various temperatures (Figure 2). A second nomograph is presented to predict the allowable fish loading to limit ammonia to 0.5 mg L^{-1} as a function of length for various temperatures (Figure 3).

B. NUTRITION AND FEEDING

Since the nutrition of experimental fish may influence results, the diet must be selected carefully. Although there are exceptions, fish should be maintained for life on the food to be used during research (Brauhn and Schoettger, 1975). Initially, commercial food will be fed

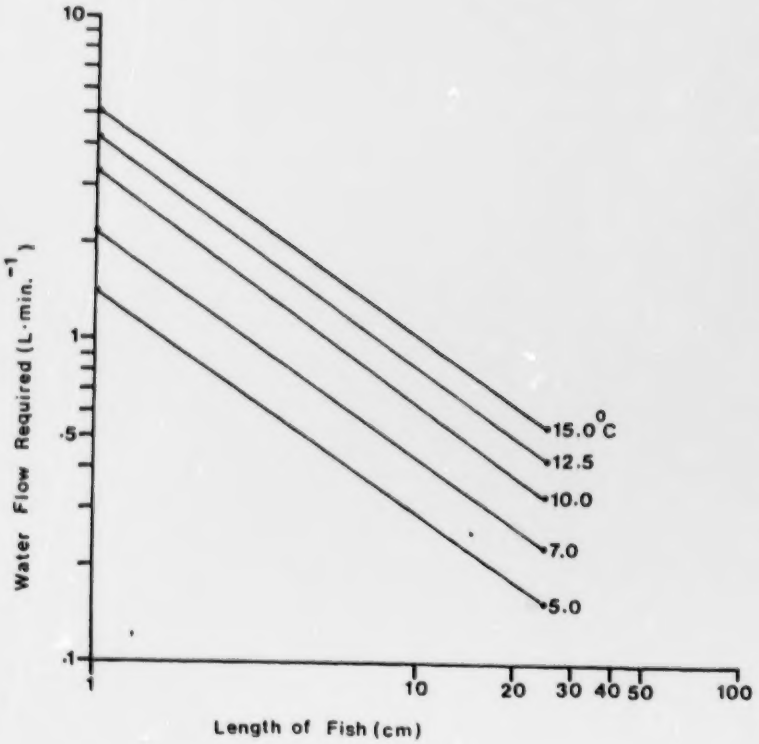
to the fish maintained at the Alberta Environmental Centre. In the future foods will be produced by the Nutrition Section. Fish will be fed 1.5-2% of their body weight per day, an amount that will maintain good condition, but limit growth. Detailed nutritional requirements are discussed in Phillips (1970), Leitritz (1959), and Subcommittee on Fish Nutrition (1973).

Pelleted fish food has been chosen for ease of feeding, storage and the elimination of diet mixing. The U.S. standards for fish food particle sizes are presented in Table 5. A starter diet will be fed to fry for 2 weeks after swim-up; fish up to 7.6 - 10.2 cm will be fed the smallest pellet (Table 5).

Fish food will be stored until required in a controlled environmental room at 4°C. Generally the maximum storage time should not exceed 60 days. Between feedings food should be kept in a dry area and not exposed to the sun. Fish should be fed slowly, by hand or with automatic feeders, at a rate that allows the fish to consume the food almost immediately, broadcasting the food over the water to distribute it to a maximum number of fish. Excess food may be consumed but some leaching of nutrients will occur (Phillips, 1970). Generally small fish will consume more of their daily ration if portions are fed several times a day. Large fish may be fed less often, down to once a day as they approach sexual maturity (Brauhn and Schoettger, 1975).

Figure 2.

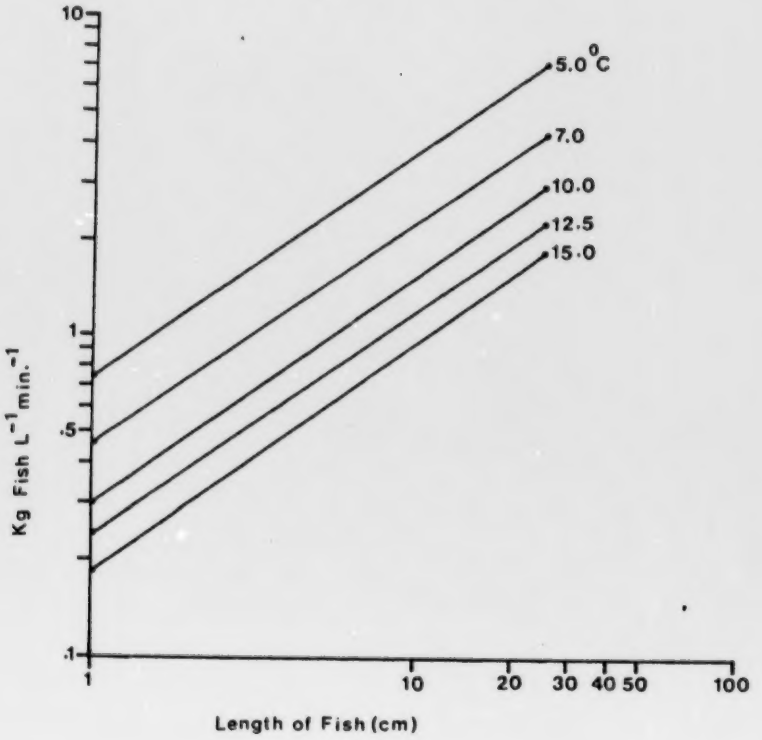
Water Flow Required Per Kg of Salmonid Fish to Limit Ammonia to 0.5mgL^{-1} as a Function of Length and Temperature.



Source: Adapted from Speece, 1973.

Figure 3.

Allowable Fish Loading To Limit Ammonia to 0.5mgL^{-1} As A Function of Length and Temperature.



Source: Adapted from Speece, 1973.

Table 5. Particle size for trout feeds specifications.

The following particle sizes are standard for contract purchases of fish feed for National Fish Hatcheries. Sizes are US Standards.

Sizes	Standard designation mesh size	Particles per gram
Starter	420-595 µm	38,800
No. 1 Granule	595-841 µm	8,580
No. 2 Granule	841 µm - 1.19 mm	2,010
No. 3 Granule	1.19 - 1.68 mm	565
No. 4 Granule	1.68 - 2.83 mm	161
Pellets, 2.4 x 2.4 mm		76.1
Pellets, 3.2 x 3.2 mm		35.6
Pellets, 4.8 x 4.8 mm		12.2
Pellets, 6.4 x 6.4 mm		5.22

Source: Adapted from Phillips, 1970.

C. LIGHTING

Fish will be maintained under controlled dusk to dawn photoperiods throughout the year. The dusk to dawn feature allows a simulation of natural conditions of sunrise and sunset, and avoids stressing the fish with a sudden change from light to dark and vice versa. The lighting sequence may be varied for research projects which require seasonal lighting patterns (Drummond and Dawson, 1970). Fish in the holding laboratory will be maintained on a 12 h light and 12 h dark sequence. Intensity at the surface of the fish tanks should range from 20 to 30 lux.

D. ROUTINE MAINTENANCE

1. Routine Checks

Parameters of dissolved oxygen, temperature, conductivity, pH and flow rate are measured twice daily. Rings and watches should be removed and hands disinfected, or polypropylene gloves worn. All equipment should be disinfected between tanks. Tanks are also checked for mortalities, dead and abnormal fish are removed immediately. See Appendix B for detailed discussion of the above parameters. Once a week, a water sample will be submitted to the Chemistry Wing for comprehensive analysis (Table 6).

Table 6. Water Quality Parameters for Analysis of Dilution Water

Test	Parameter	Test	Parameters
Routine	pH	Metals	Cobalt
	Conductivity		Copper
	Calcium		Nickel
	Magnesium		Cadmium
	T-Hardness		Lead
	Sodium		Zinc
	Potassium		Manganese
	Iron		Aluminum
	Silica		Arsenic
	$(NO_2 + NO_3)$-N		Selenium
	Nitrite-N		Chromium
	Chloride		Vanadium
	Sulphate		Molybdenum
	Fluoride		
	Carbonate		
	Bicarbonate		
	T-Alkalinity		
	Total Dissolved Solids		

E. BEHAVIOUR

Day to day observations are a valuable tool in assessing fish condition. Abnormal behavior may be the first indication of stress-producing factors. Indicators of stress factors that may influence experimental results include feeding response, swimming posture and unusual or erratic movements. Table 7 presents some stress factors and their possible causes.

Table 7. Cause-related Behavior in Fish.
Several Observations May be Made Simultaneously and May be caused by a Combination of Two or More Problem Areas.

Observation	Possible problem
1. Surfacing and swimming with mouth half emerged - a,b,c,d	a. insufficient oxygen; toxic chemicals; high NH_3
2. Scraping against sides of tanks - a/ b,d,f	b. parasitism of skin or gills.
3. Erratic swimming - a,b,c	c. rapid temperature changes; parasitism of nervous system; virus disease
4. Crowding around water inflow - a, b	d. parasitism of intestine; bacterial disease
5. Distending abdomen and difficulty in swimming - d, e, f	e. diet consistency
6. Refusal to feed actively - a, c, d, e, f	f. nutrition deficiency
7. Listlessness, emaciation - b, c, d, e, f	

a/Normal fathead minnow courtship behavior includes coloration change and display near tank sides.

Source: Brauhn & Schoettger (1975)

V. LABORATORY MAINTENANCE

The following quality control measures will be taken as part of normal fish maintenance:

a) Each tank will be provided with separate labelled dip nets. Nets will be disinfected in hibitaine and rinsed with clear water after each use.

b) No hand makeup or hand cream will be worn. Jewelry should be removed, and hands and arms washed with a disinfectant soap and rinsed with clear water, or polypropylene gloves worn prior to feeding fish, or if it is necessary to reach into a tank for any reason. Hands or gloves will be disinfected between tanks.

c) All instruments should be disinfected with hibitaine and rinsed with clear water between tanks. Instruments will not be interchanged between laboratories.

d) All dead fish will be disposed of by incineration.

e) Daily maintenance of the tanks will be required. Attached material should be dislodged with a disinfected rubber spatula or abrasive sponge and siphoned off (American Public Health Assoc. 1975). Fish should be disturbed as little as possible when tanks are cleaned. Frequent cleaning decreases BOD and in turn increases carrying capacity (Klontz, Brock and McNair, 1978). Tanks should be completely disinfected with hibitaine or a similar type disinfectant between the introduction of batches of fish and eggs.

f) Experimental tanks and apparatus should be disinfected with hibitaine and rinsed with clear water at the end of each experiment.

g) Contaminated beakers or flasks will be rinsed with water and cleaned

with an appropriate material for removing the toxicant.

Nalgene or plastic: use biodegradable soap (Sparkleen); rinse with deionized water 3 - 4 times. For severe contamination discard the item.

Glassware: may be sterilized in the autoclave or disinfected.

Disinfection: use dichromate cleaning solution (15 g powdered potassium or sodium dichromate to 500 ml conc. H_2SO_4. Prepare and store in fumehood). The vessel to be cleaned is lowered into the cleaning solution with safety tongs, swirled and rinsed. If the item is heavily soiled, it may be left in the solution until it is clean. The solution is expired when the color changes from orange to green. All glassware should be rinsed with clear water.

h) Routine calibration and maintenance checks will be performed on the following instruments: pH meters and electrodes, ammonia meters and electrodes, conductivity meters and electrodes, dissolved oxygen meters and probes, hydrolab meters and probes. Manuals are available for all meters. See Table 8 for schedules. Fresh solutions for calibration procedures will be made up as required.

i) The laboratories will be kept clean and tidy at all times.

j) No food or drinks will be allowed in the laboratories.

k) Records will be maintained for each population in each tank.

Table 8. Calibration Schedule For Analytical Instruments

Instrument or Electrode	Type of Calibration	Before use	1 Week	2 Weeks	3 Weeks	4 Weeks	As Necessary
pH meter	complete					X	
	To buffer	X					
pH electrode	complete	X					
	To buffer	X				* condition	
ammonia meter	complete	X					
ammonia electrode	complete	X					change membrane at start of new experiment
conductivity meter	complete	X					
conductivity electrode	redline	X					
dissolved oxygen meter	complete	X					
dissolved oxygen probe	complete	X					change membrane between experiments
hydrolab meter & probes	complete	X	X				

* Condition the pH electrode by leaving it in .1M HCL for 2 hours. Rinse and recalibrate the pH meter.

VI. BIOASSAY PROCEDURES

The Water Quality Control Branch of Alberta Environment routinely collects liquid effluents from Alberta industries that discharge into surface water. A chemical analysis and 96 hr static bioassay of the whole liquid effluent are performed by staff at the Alberta Environmental Centre.

A. SAMPLING PROCEDURES

1) Clean twenty-five gallon (114L), polyethylene lined barrels are used. In order to reduce the possibility of contamination, similar industry types have been grouped and assigned a color relating sample barrel colors to industry type (Table 9). After use, the barrels are rinsed thoroughly with hot water, and depending on the industry sampled, a mild acid wash may be necessary, followed by thorough rinsing with tap water. Clean barrels are stored at the Alberta Environmental Centre, and sent to the Pollution Control Division offices in Edmonton and Calgary as required.

2) Twenty-five or fifty gallons (114,228L) of sample are collected, depending on the size of fish to be used, and the exposure volume required. The barrel should be rinsed with effluent at the time of collection. Barrels are filled to the top, and any air bubbles are forced out. The barrel is sealed and returned to the laboratory as soon as possible.

Table 9. Effluent Categories Monitored by the Aquatic Bioassay Section Barrel color coding for bioassay sampling is also shown.

	Barrel Color
Industry Type: Building Products	Blue
Canada Cement Lafarge Ltd. - Exshaw	
Domtar Chemicals Ltd. - Cochrane	
Fiberglass Canada Ltd. - Edmonton	
Industry Type: Chemical Products	Blue
Alberta Sulphate Ltd. - Horseshoe Lake	
Johns Manville Canada Inc. - Fort Saskatchewan	
Celanese Canada Ltd. - Edmonton	
Uniroyal Chemicals Ltd. - Edmonton	
Alberta Gas Ethylene Co. Ltd. - Joffre	
Dow Chemical of Canada Ltd. - Fort Saskatchewan	
Two Hills Chemical Co. Ltd. - Duvernay	
Industry Type: Gas Plants (Class C)	Red
Shell Canada Resources Ltd.	
Shell Jumping Pound - 15 M W of Calgary, 5-7 M SW of Cochrane	
Shell Waterton - South of Pincher Creek	
Western Decalta Petroleum Ltd. - Turner Valley	
Diamond Shamrock Alberta Gas Ltd. - Fort Saskatchewan	
Industry Type: Oil Refineries	Red
Gulf Oil Canada Ltd.	
Gulf Oil Calgary	
Gulf Oil Edmonton	
Imperial Oil Ltd. - Calgary Bulk	
Imperial Oil Enterprises Ltd. - Edmonton	
Shell Canada Ltd. - Bowden	
Texaco Canada Ltd. - Edmonton	

Table 9 continued

Industry Type: Oil Sands	Red
Suncor Inc. Resources Group - Fort McMurray	
Industry Type: Fertilizer Industries	Green
Canadian Industries Ltd. - Edmonton	
Esso Chemical Canada - Redwater	
Sherritt-Gordon Mines Ltd. - Fort Saskatchewan	
Western Co-operative Fertilizers Ltd. - Medicine Hat	
Canadian Fertilizers Ltd. - Medicine Hat	
Cominco Ltd.	
Cominco Ltd. - Carseland	
Cominco Ltd. - Calgary	
Industry Type: Food and Beverage	Green
The Canadian Salt Co. Ltd. - Lindbergh	
Alberta Sugar Co. Ltd. - Taber	
Alberta Food Products Ltd.- Edmonton	
Industry Type: Pulp and Paper	Green
Proctor and Gamble Co. of Canada Ltd. - Grande Prairie	
St. Regis (Alberta) Ltd. - Hinton	
Industry Type: Iron and Steel	Yellow
The Steel Co. of Canda Ltd. - Edmonton	
Industry Type: Thermal Power	Yellow
Alberta Power Ltd. - Battle River	
Calgary Power Ltd. - Lake Wabamum	

B. BIOASSAY TEST

The bioassay consists of five concentrations of effluent (20, 40, 60, 80, 100%) plus a control. The day that the sample is to be collected the following preparaions should be made:

1) Six polyethylene bags must be rinsed (3 times) with dilution water to remove any contaminants from the bags. The bags are checked for holes. Each bioassay container is lined with a polyethylene bag to prevent cross-contamination between bioassays.

2) The dilution water is poured using a clean calibrated polyethylene pail reserved for that purpose. The appropriate volumes are given in Table 10, based on either twenty or forty litre volumes of test solution.

3) Regardless of whether the bioassay is to be conducted in a water bath or a controlled environmental room, the temperature is set to 15°C ± 1°C.

4) When the effluent arrives at the laboratory, it should be poured immediately. A clean calibrated polyethylene pail reserved for effluent is used to pour the appropriate dilutions (Table 10). At this time a sample is collected from the barrel, and another of the dilution water, for chemical analysis by the Chemistry Wing. Parameters to be analysed may vary for each industry group (Appendix C).

5) The following chemical parameters of the 100% effluent are recorded immediately: temperature, conductivity, pH, dissolved oxygen, and ammonia.

If the dissolved oxygen is below 7 mg L^{-1}, the bioassay should be pre-aerated for not more than two hours at a rate of 5.0-7.5 cm^3 $min^{-1}.L$ prior to starting the test; if volatiles are present, aeration should be

carried out with closed bags to minimize the loss of these chemicals. If the dissolved oxygen is 7 mg L^{-1} or greater, the bags are sealed, and the sample left overnight to acclimate to the test temperature of 15°C. An aeration rate of 5.0 - 7.5 cm^3 min^{-1}.L should be maintained in the bioassay containers for the duration of the test (Environment Canada, 1980).

If pH is <6.5 or >8.5, pH must be adjusted with either 0.1 N sodium hydroxide or 0.1 N sulphuric acid.

If the initial ammonia (NH_3-N) level is >1 mg L^{-1}, the ammonia reading should be taken every twenty-four hours to monitor the loss of ammonia due to air stripping. Ammonia values may be converted to unionized NH_3 at the test temperature and pH.

The bioassay should be started at 0900 h, the day following collection of the sample. Mortality of the fish stock for four days prior to the start of the bioassay should be less than 5% (Sprague, 1973). Generally ten fish (with minimum of 5 fish) per container are used, depending upon fish size and sample volume. Individual fish should weigh from 0.5 to 10g, and within a test group the length of the largest fish should not exceed two times the smallest in the same container. For every one gram of fish, there should be at least 0.5 L of bioassay sample or control water for each 24 hours of the test. The fish are not fed for two days prior to the bioassay, or during the test. This stabilizes their metabolic rate, and minimizes organic matter build-up in the test vessels.

Table 10. Effluent Dilutions for Twenty and Forty Litre Bioassay Tests

Total Test Volume Required (L)	Desired Concentration %	Volume of Dilution Water (L)	Volume of Effluent (L)
20	100	0	20
	80	4	16
	60	8	12
	40	12	8
	20	16	4
	control	20	0
40	100	0	40
	80	8	32
	60	16	24
	40	24	16
	20	32	8
	control	40	0

Chemical parameters of each effluent concentration plus the control are measured at the start of the bioassay and monitored at 24 hour intervals for the duration of the test. Parameters measured are temperature, conductivity, pH, dissolved oxygen, and ammonia if necessary.

Test fish are randomly selected (using cards, dice or random tables) and added to the test chambers as quickly as possible and with as little duress as possible.

Mortality at the start of the bioassay and throughout the day must be recorded. Observation times are based on a linear series, 0 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, 72 hours, and 96 hours. Depending upon the response of the fish, the frequency of checks may vary. Fish are considered dead when there is no respiratory or other movements, and no response to gentle prodding. Dead fish should be removed immediately (or at least once a day) and the fork lengths and wet weights recorded. Mortality in the control tank should not exceed 10% for the duration of the experiment. At the end of the bioassay, all remaining fish should be sacrificed using tricaine methane sulfonate (MS222). Fork lengths and wet weights should be recorded. All data should be recorded on the appropriate data sheets for subsequent analysis (Appendix D).

C. CHEMICAL REFERENCE TOXICANT BIOASSAY

Whenever a 96 h static bioassay is performed, it is necessary to have some measure of sensitivity of the fish stock being tested. This is accomplished by running a chemical reference toxicant bioassay in conjunction with the industrial bioassay.

Phenol is a systemic toxicant which affects many physiological systems in fish, for that reason, it has been chosen as the reference toxicant in our laboratory. A 24 h static bioassay is performed using concentrations of 5, 8, 10, 12, and 15 mg L^{-1}. A normal 24 h LC_{50} occurs between 8 and 12 mg L^{-1}. The appropriate dilution volumes are calculated using the following equation:

Volume of stock solution required (ml) =

$$\frac{\text{1000 ml [desired concentration (mg } L^{-1})]}{\text{stock concentration (mg } L^{-1})}$$

Generally a 3000 ppm stock solution is prepared (3000 ppm = 3000 mg L^{-1}).

Desired Concentration (mg L^{-1})	ml stock 20 L^{-1}	ml stock 40 L^{-1}
5	33.33	66.67
8	53.33	106.67
10	66.67	133.33
12	80.00	160.00
15	100.00	200.00

Chemistry and mortality checks of the phenol bioassay should occur at the same frequency as for the industrial bioassay, and be recorded on the data sheets for analysis with the 96 h static bioassay results.

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VIII. APPENDIXES

APPENDIX A

Fish Health Protection Regulations

Fish must be free of disease(s) categorized in schedule B,C, and D.

Schedule B

Diseases or Disease Agents Found in Live Fish or Their Source

1. Any filterable replicating agent capable of causing cytopathic effects in the cell lines of fish specified by the Minister, including but not limited to
   a) Viral Hemorrhagic Septicemia (Egtved) (Egtved virus, VHSV)
   b) Infectious Hematopoietic Necrosis (IHNV)
   c) Infectious Pancreatic Necrosis (IPNV)
2. Whirling Disease (Myxosoma cerebralis)
3. Ceratomyxosis (Ceratomyxa shasta)
4. Furunculosis (Aeromonas salmonicida)
5. Bacterial Kidney Disease (KD bacterium)
6. Enteric Redmouth Disease (RM bacterium)

SCHEDULE C

Diseases or Disease Agents Found in Dead Fish or Their Source

1. Viral Hemorrhagic Septicemia (Egtved) (Egtved virus, VHSV)
2. Whirling Disease (*Myxosoma cerebralis*)

SCHEDULE D

Diseases or Disease Agents Found in Live Fish or Their Source

1. Myxobacterial infections
2. Motile Aeromonad Septicemia (motile *Aeromonas* sp)
3. Pseudomonad Septicemia (*Pseudomonas* sp)
4. Vibriosis (*Vibrio* spp)

From: Fish Health Protection Regulations
Manual of Compliance
Dept. of Fisheries and the Environment
Fisheries and Marine Service
Ottawa, Dec./76

APPENDIX B

Water Quality Parameters for Routine Checks

Dissolved Oxygen:

Salmonids require a minimum dissolved oxygen content of 5 mg L^{-1} at 15°C or approximately 50% saturation (Davis, 1975). At this level growth is marginal. Optimal growth occurs when the water is 95% saturated with oxygen, with both temperature and altitude affecting the oxygen carrying capacity of the water (Klontz, Brock and McNair, 1978).

Temperature:

The preferred water temperature for fish is referred to as SET or standard environmental temperature. SET for rainbow trout is estimated at 15°C. At this temperature, trout can increase length by nearly 4.0 cm mo^{-1}. For each 1°C decrease in temperature there is a 9% decrease in metabolic rate, and a corresponding decrease in metabolic intake and waste product output (Klontz, Brock and McNair, 1978). Water temperatures between 0-9°C cause sluggishness and reduction of appetite, while temperatures above 13°C stimulate appetite and growth. Temperatures above 17-20°C should be avoided, and 25°C approximates the upper lethal limit (Brauhn and Schoettger, 1975).

Appendix B continued

Conductivity:

Incoming Vegreville water has a conductivity of about 1000 microsiemens cm^{-1} before treatment. Treatment will reduce this value to some degree. Conductivity should not vary more than 10% to be considered constant (American Public Health Assoc. 1975). If conductivity increases suddenly, this is an indication of increased dissolved anions and cations, and thus a change in water quality. Conversely, if conductivity drops suddenly this is probably a result of precipitations of anions and cations. When the Edmonton pipeline is complete, conductivity will range from about 100 microsiemens cm^{-1} to about 175 microsiemens cm^{-1} based on past Edmonton water chemistry data.

pH:

pH in the holding tanks should be in the range of 7.0 - 8.5. When the pipeline from Edmonton is complete, and Edmonton water is being treated, it may be necessary to bubble carbon dioxide into the lines to lower pH.

Flow Rate:

The flow rate should be maintained at a calculated rate to prevent over loading of the tanks. (Figure 2).

APPENDIX C

Recommended Chemical Analysis for Industry types

ROUTINE

PARAMETER	IRON & STEEL	THERMAL POWER	OIL SANDS	OIL REFINERIES	FOOD & BEVERAGE	PULP & PAPER	GAS PLANTS	CHEMICAL FERTILIZERS	CHEMICAL PRODUCTS	BUILDING PRODUCTS
pH	X	X	X	X	X	X	X	X	X	X
Conductivity (Cond.)	X	X	X	X	X	X	X	X	X	X
Calcium	X	X	X	X	X	X	X	X	X	X
Magnesium	X	X	X	X	X	X	X	X	X	X
Total Hardness (T-Hardness)	X	X	X	X	X	X	X	X	X	X
Sodium	X	X	X	X	X	X	X	X	X	X
Potassium	X	X	X	X	X	X	X	X	X	X
Iron	X	X	X	X	X	X	X	X	X	X
Silica	X	X	X	X	X	X	X	X	X	
$(NO_2 + NO_3)$-N	X	X	X	X	X	X	X		X	
Nitrite-N	X	X	X	X	X	X	X		X	
Chloride	X	X	X	X	X	X	X		X	
Sulphate	X	X	X	X	X	X	X		X	
Fluoride	X	X	X	X	X	X	X		X	
Carbonate	X	X	X	X	X	X	X		X	X
Bicarbonate	X	X	X	X	X	X	X		X	X
Total Alkalinity (T-Alkalinity)	X	X	X	X	X	X	X		X	X
Total Dissolved Solids (TDS)	X	X	X	X	X	X	X		X	

APPENDIX C continued

	PARAMETER	IRON & STEEL	THERMAL POWER	OIL SANDS	OIL REFINERIES	FOOD & BEVERAGE	PULP & PAPER	GAS PLANTS	CHEMICAL FERTILIZERS	CHEMICAL PRODUCTS	BUILDING PRODUCTS
GENERAL 1	Biological oxygen demand (BOD)					X	X				
	Odor							X			
	Total residue (TR)										
	Total residue filterable (TRF)										
	Non-filterable residue (NFR)		X	X	X	X	X	X	X		X
	Non-filterable residue filtered (NFRF)										
	Turbidity										
	Surfactants			X							
	Tannins and lignins (T&L)						X				
	Cr^{+6}	X	X	X	X		X	X	X	X	X
	Color						X				
GENERAL 2	Chemical oxygen demand (COD)							X			
	NH_3-N			X	X			X	X		
	Phosphate-Total								X		
	Total kjeldahl nitrogen (TKN)			X		X					
	Ortho-phosphate ($O\text{-}PO_4$)										
CARBON	Total organic carbon (TOC)				X						
	Total carbon (TC)										
	Total inorganic carbon (TIC)										

APPENDIX C continued

	PARAMETER	IRON & STEEL	THERMAL POWER	OIL SANDS	OIL REFINERIES	FOOD & BEVERAGE	PULP & PAPER	GAS PLANTS	CHEMICAL FERTILIZERS	CHEMICAL PRODUCTS	BUILDING PRODUCTS
NON-ROUTINE	Dissolved oxygen (DO)										
	Oil & grease		X	X	X		X	X		X	
	Phenols		X	X	X			X	X	X	
	Cyanide			X	X				X		
	Sulphide		X	X	X			X			
	Mercury						X				
	Chlorine										
	METALS: Dissolved Total										
GROUP 1	Cobalt									X	
	Copper	X	X	X	X	X	X	X	X	X	
	Nickel	X	X	X	X	X	X	X	X	X	
	Cadmium	X	X	X	X	X	X	X	X	X	
	Lead	X	X	X	X	X	X	X	X	X	
	Zinc	X	X	X	X	X	X	X	X	X	
GROUP 2	Arsenic							X			

APPENDIX D

Bioassay Data Sheets

ALBERTA ENVIRONMENTAL CENTRE
AQUATIC BIOLOGY SECTION
VEGREVILLE, ALBERTA

BIOASSAY DATA SHEET

COMPANY	TYPE OF TEST	TEST #
SOURCE	MATERIAL TESTED	DATE SAMPLED
PLANT TREATMENT	AMOUNT OF EFFLUENT RECEIVED	DATE RECEIVED
STATUS OF PLANT	DILUTIONS	DATE TESTED TIME
SAMPLED BY	TEST ORGANISM LOCATION	SEND RESULTS TO

BIOASSAY DATA SHEET II

TEST NUMBER: _____ TEST DATE: _____

PARAMETERS	CONCENTRATIONS (MEAN & STANDARD DEVIATION)					
	CONTROL					
TEMPERATURE (^{o}C)						
DISSOLVED OXYGEN (mg L^{-1})						
pH						
CONDUCTIVITY (microsiemens)						
AMMONIA (mg L^{-1})						
FISH LENGTH (cm)						
FISH WEIGHT (gm)						
LOADING DENSITY ($L\,g^{-1}$)						
NUMBER FISH / DILUTION						
NUMBER DILUTIONS / CONC						
VOLUME OF DILUTIONS (L)						
MST_{50} (Hr.)						
LC_{50} (Conc by Vol)						

TOTAL _____

REMARKS:

Test Date: _____ Test Number: _____

BIOASSAY DATA SHEET III

EFFLUENT CONCENTRATION	REPLICATE HOURS	NUMBER OF MORTALITIES	% DEATH

Species BIOASSAY CHEMICAL DATA SHEET TEST NO. _____ Test Date _____

Time \ Conc	Control																													
	DO	pH	Temp	Cond	NH_3	DO	pH	Temp	Cond	NH_3	DO	pH	Temp	Cond	NH_3	DO	pH	Temp	Cond	NH_3	DO	pH	Temp	Cond	NH_3	DO	pH	Temp	Cond	NH_3

(53)

BIOASSAY MORTALITY DATA SHEET

STARTING DATE OF TEST _____ TEST ORG. _____

ENDING DATE OF TEST _____ TEST # _____

CONCENTRATION	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
	CONTROL																		
TIME																			OBSR
0900																			

BIOASSAY LENGTH/WEIGHT DATA SHEET

STARTING DATE OF TEST _____ TEST ORG. _____

ENDING DATE OF TEST _____ TEST # _____

CONCENTRATION CONTROL

	Lgth/wght		Lgth/Wght		Lgth/Wght		Lgth/Wght		Lgth/Wght		Lgth/Wght		Lght/Wght		Lgth/Wght		Lgth/Wght	
TIME																		

Alberta Environmental Centre
Aquatic Biology

AQUATIC BIOLOGY - FISH BIOASSAY SUMMARY

TYPE OF BIOASSAY _____ SOURCE OF SAMPLE _____ DATE OF SAMPLE _____ DATE OF TEST _____

TEST SPECIES _____ STORAGE _____ AERATION _____

% Concentration	Mean Survival Time (M.S.T.)	TIME - CUMULATIVE % MORTALITY													Average D.O.	Average Temp.	Average pH	Average Fish Length	Average Fish Weight	Average NH_3 Conc.	No. of Fish Tested	Hour LC_{50}
		1/4	1/2	1	2	4	8	12	24	36	48	60	72	84	96								
CONTROL																							
STANDARD REFERENCE TOXICANT																							